THE JOURNAL OF INVESTIGATIVE DERMATOLOGY
Vol. 52, No. 6

Printed in U.S.A.

THE ULTRASTRUCTURE OF *TRICHOPHYTON* AND A DOUBLE CELL WALL IN THE HYPHA*

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ABSTRACT

Many hyphae with double structures consisting of an outer and an inner hypha were demonstrated with an electron and a light microscope when microconidia of *Trichophyton mentagrophytes* were cultured with shaking in a Sabouraud's broth for 96 hours.

It seems that this double structure results from the extension of a septum of the hypha through the interior to form a new "inner" hypha enclosed in the old "outer" hypha.

Recent electron microscopic studies have revealed the ultrastructure of dermatophytes (1–7). However, no detailed reports are yet available concerning the process of the budding of spores and structural alterations of the fungi in relation to the growth of hyphae.

Using the electron microscope, we observed in many hyphae, in the process of development from spores, a double cell wall structure. This hitherto undescribed finding will now be reported together with light microscopy observations.

MATERIALS AND METHODS

The strain of *Trichophyton mentagrophytes* maintained in our department, was cultured on Sabouraud's dextrose agar at 27° C for 2 weeks. The culture was harvested after sufficient growth and a fungal suspension was prepared by addition of distilled water after pulverization of the growth with mortar and pestle. A homogeneous suspension of microconidia without contamination of hyphae was obtained by filtration of this suspension through a sterile cotton filter apparatus prepared by placing approximately 0.5 g of defatted cotton over the small hole at the apex of a centrifuge tube. Flasks containing 200 ml of Sabouraud's broth were inoculated with several drops of this suspension of spores. After 96 hours of culture under shaking at 27° C, hyphae were collected by centrifugation and immediately fixed with 2% buffered osmium solution (pH 7.4) for 3 hours without prior washing with water. Dehydration was carried out with an acetone system, starting from the concentration of 35%, and the specimen was embedded in Epon. Ultra-thin sections were prepared with a Porter-Blum microtome. After 3 hours of uranium staining, the specimen was observed with a Hitachi HU-11 A type electron microscope and pictures were taken. Another specimen processed in exactly the same manner was sectioned in approximately 2 μ thickness and stained with PAS, without removing resin, for microscopic observation.

Received October 11, 1968; accepted for publication December 3, 1968.

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RESULTS

A. Electron Microscopic Findings

After 96 hours of incubation, the developed hypha, especially in the segment near the apex, consisted of cells with abundant cytoplasm, many mitochondria, one or two nuclei and endoplasmic reticula (Fig. 1). Among these normal hyphae, there was frequent occurrence of a hypha containing another hypha in the interior (Figs. 2–6). Since two layers of cell wall have been found, we call it "double hyphae," the hypha inside being called "inner hypha," the one outside, "outer hypha." Each of these will be explained in the section to follow.

1. Outer hypha, OH. The external wall of the hypha (outer cell wall, OCW) including the outside portion of the internal cell wall (inner cell wall, ICW) has been named "outer hypha (OH)," and shows no discernible internal structure. In some portion, coalescence or conjunction of the external and the internal cell wall is seen (Figs. 2, 3, 4, 6). Cytoplasm in general shows some degree of degeneration and concentration. Although the organelles such as nuclei, mitochondria, endoplasmic reticula and cellular membrane are not distinct, occasionally a few mitochondria and vacuoles are noted as in Figure 5 which probably represents an early stage of degeneration and concentration.

2. Inner hypha, IH. The inner hypha borders the cytoplasm of the outer hypha along the internal cell wall. The cell wall is electron transparent as is the outer hypha and the structure is not distinct. The plasma membrane (PM) is seen as a unit membrane which has occasional interruptions and is rich in undulations and depressions into the cytoplasm, and sometimes gives the appearance of vesicules.

The stroma of cytoplasm gives a granular appearance (Fig. 2). Nuclei (N) (Figs. 3 and 4),

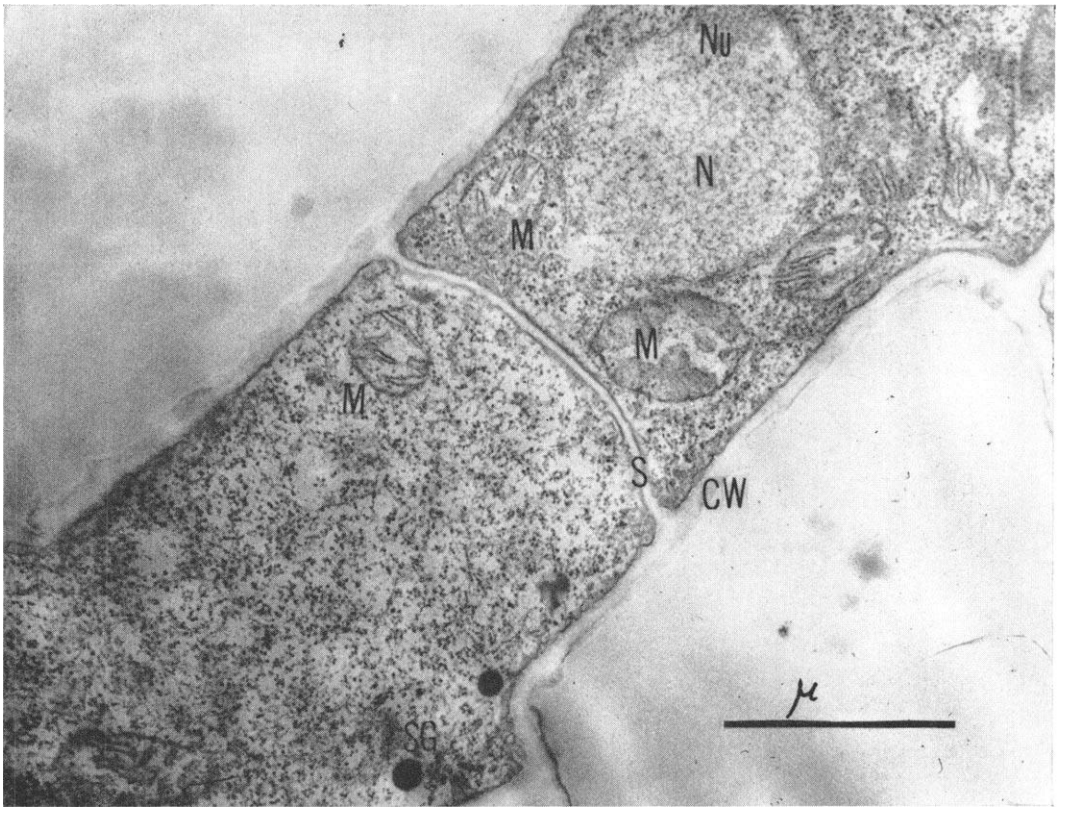

FIG. 1. Normal hypha. The nucleus (N) with nucleolus (Nu) and many mitochondria (M) are observed. S indicates septum; SG, septal granule.

the areas of a rather low electron density, are bordered by two layers of nuclear membrane (NM) from the surrounding stroma of the cell. The stroma of nuclei appears finely granulated. The nuclear membrane is interrupted by structures probably representing nuclear pores (NP) (Fig. 3). Findings suggesting continuation of the nuclear membrane with endoplasmic reticulum (ER) have also been obtained (Fig. 4).

There are many mitochondria (M) (Figs. 2–6). The shape is spherical or filamentous probably depending upon the direction of the cutting. Although the size is rather variable, most of them measure 0.2–0.8 μ along the long axis. Cristae of the mitichondrion appear lamellar and the bordering membrane appears as a unit membrane. In many instances, the outer layer of the bordering membrane is lacking, assuming the form of the so-called open cristae.

The endoplasmic reticulum appears as a tubular or vesicular structure (Figs. 2, 4, 6).

Septal granules (SG) are observed as an electron-dense small spherical structure, to be clearly distinguished from lipid granules (L), which usually show a slight variation in electron density, size, and shape (Fig. 2).

B. Light Microscopic Findings

Under optical microscope, the development and extension of the inner hypha inside the outer hypha has also been observed (Fig. 7). As shown in these longitudinal sections of the hyphae, one end of the inner hypha is in contact with the septum of the outer hypha (Fig. 7, A and B). The outer hypha thus gives the appearance of the sheath of the inner hypha, in good agreement with the electron microscopic finding shown in Figure 3. In Figure 7 D, a transection of the double structure is seen in its beginning stage, which may correspond to Figure 5.

DISCUSSION

The inner hypha showed the distinct ultrastructure of a typical hypha, and under the

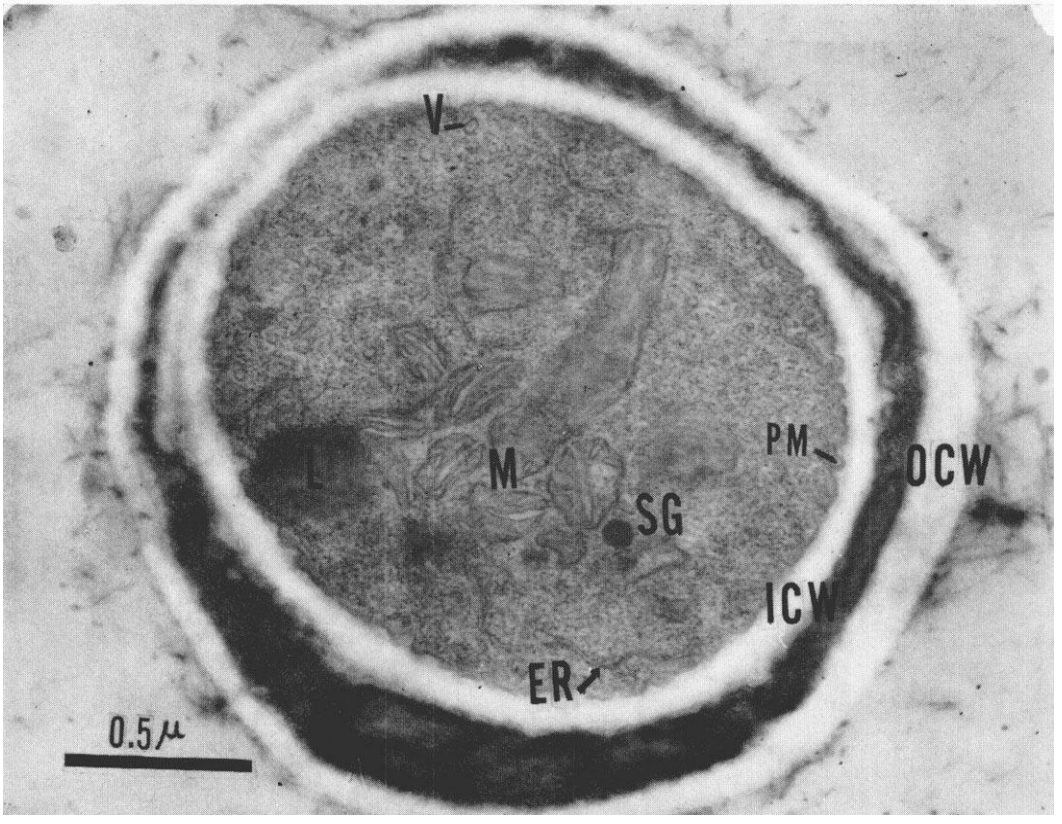

FIG. 2. A transection of the double structure of a hypha. The inner hypha borders with the cytoplasm of the outer hypha by the inner cell wall (ICW). The plasma membrane (PM) of the inner hypha shows occasional interruptions. The cytoplasm of the outer hypha is concentrated and no intracellular organelles are observed. In some portion, coalescence of the inner and the outer cell wall (OCW) is seen. SG indicates septal granule; V, vacuole; ER, endoplasmic reticulum; L, lipid granule.

optical microscope, the structure, presumably the cell wall, stained positively with PAS as did the outer hypha. Evidently, therefore, this is not an artifact.

Urabe (2) observed a double structure of hypha in the electron microscopic picture of *Trichophyton gypseum* and described it using the term "mother and daughter" cells, with the presumption that it represented spore formation. Such double structure has been found not only in the culture obtained with shaking but also in the aerial hyphae grown on Sabouraud's agar media, and probably represents the same phenomenon. According to Hachisuga (8), bacilli with endospores such as *Clostridium* assume a double structure during the process of budding. However, this appears to be different from the double structure in true fungi described by us.

Two mechanisms are conceivable for the development of inner hyphae. The first may be represented by the finding in Figure 5. When one of the cells of the hypha loses its activity for some reason, mild degeneration of cytoplasm occurs. Then, in a portion of the cell, an inner hypha develops as a compensatory mechanism and gradually expands so that one end coalesces with the separating wall while the other end continues to expand to become an inner hypha with a complete interior as seen in Figure 6. Under optical microscopy, coalescence of one end of the inner hypha with the separating wall of the outer hypha also was suggested (Fig. 7, indicated by an arrow). However, it is difficult to conceive of a new cell developing in the interior of a cell which has once lost its activity, although a similar phenomenon could possibly take place in bacteria and fungi with endospores.

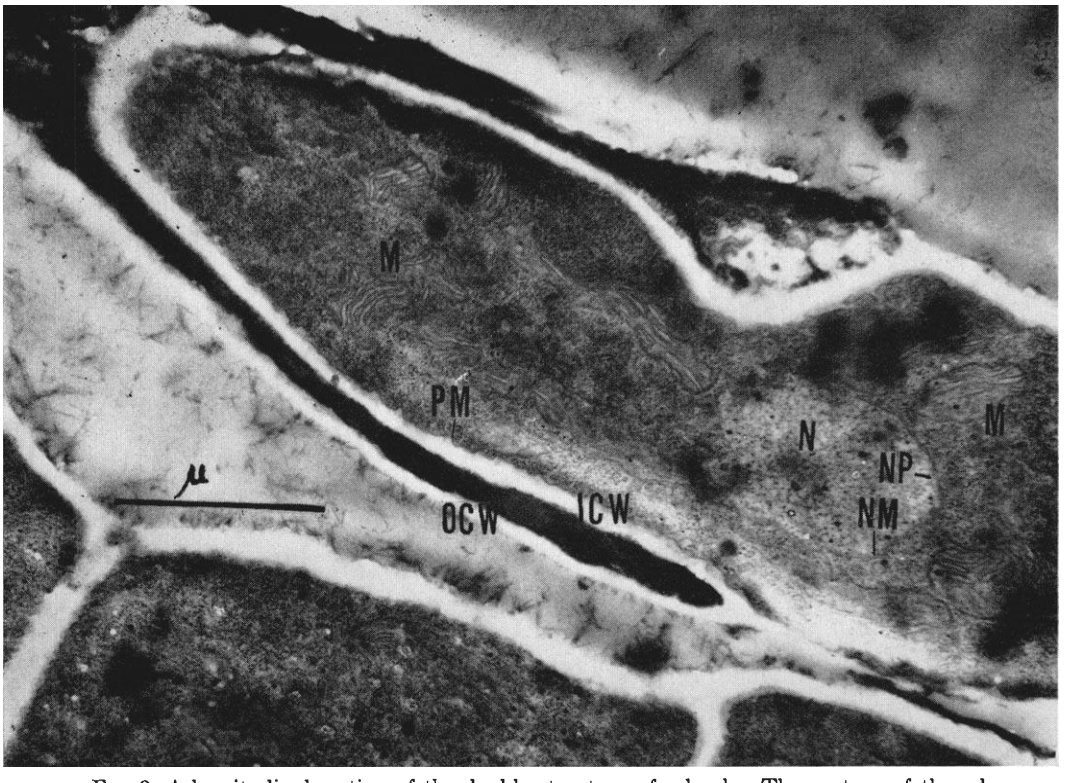

FIG. 3. A longitudinal section of the double structure of a hypha. The septum of the adjacent cell extends through the interior of the outer hypha, changing itself into the cell wall of the inner hypha. The cytoplasm of the outer hypha is concentrated and no organelles are found. NM indicates nuclear membrane; NP, nuclear pore.

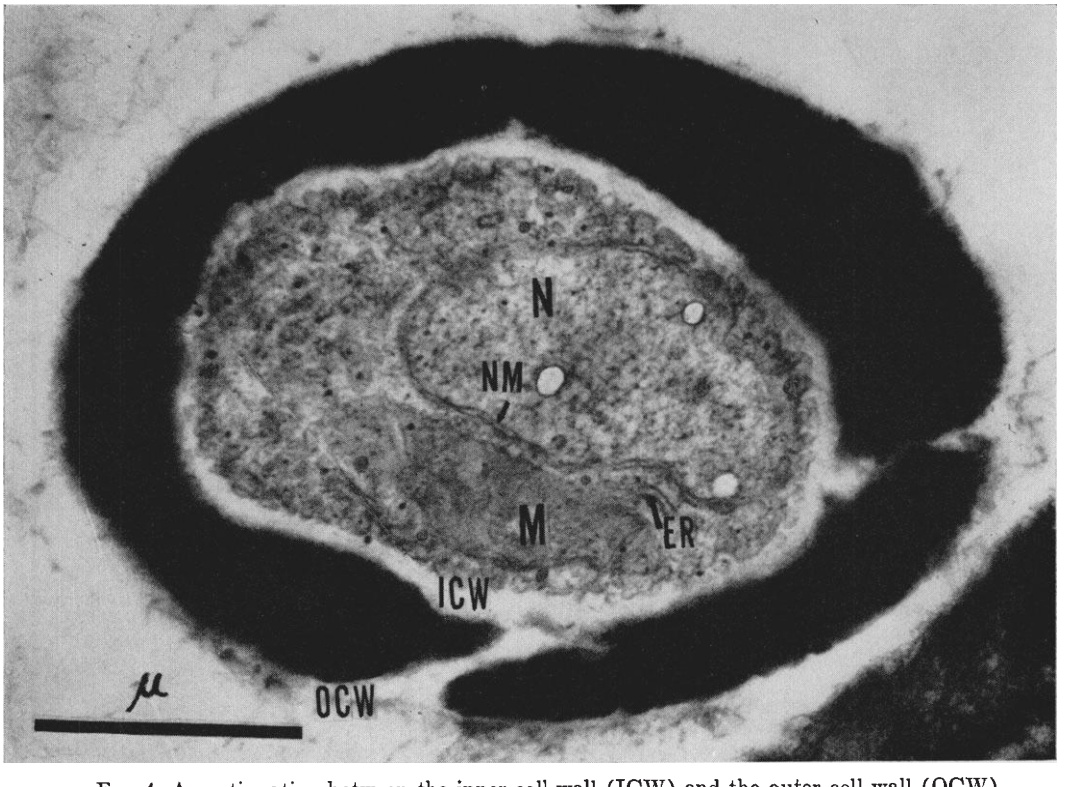

FIG. 4. A continuation between the inner cell wall (ICW) and the outer cell wall (OCW) is seen. The cytoplasm of the outer hypha is concentrated and appears structureless.

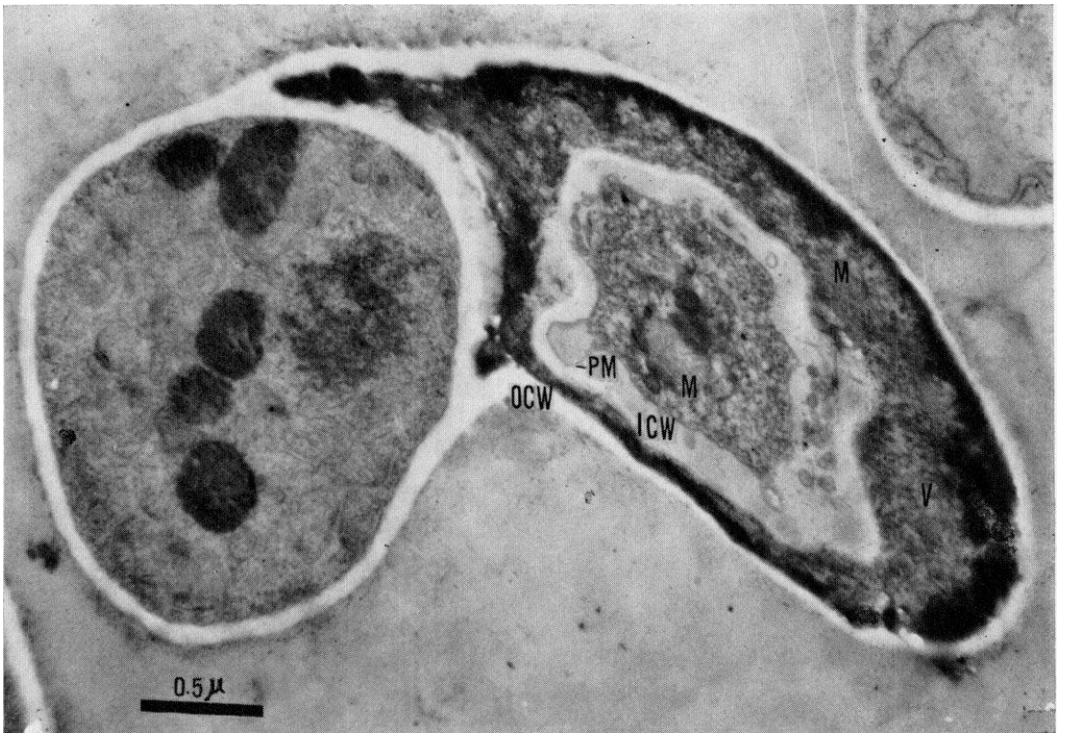

FIG. 5. An early stage in the development of the inner hypha. In the cytoplasm of the outer hypha, mitochondria (M) and vacuoles (V) still remain and a concentration of the cytoplasm is observed only near the plasma membrane.

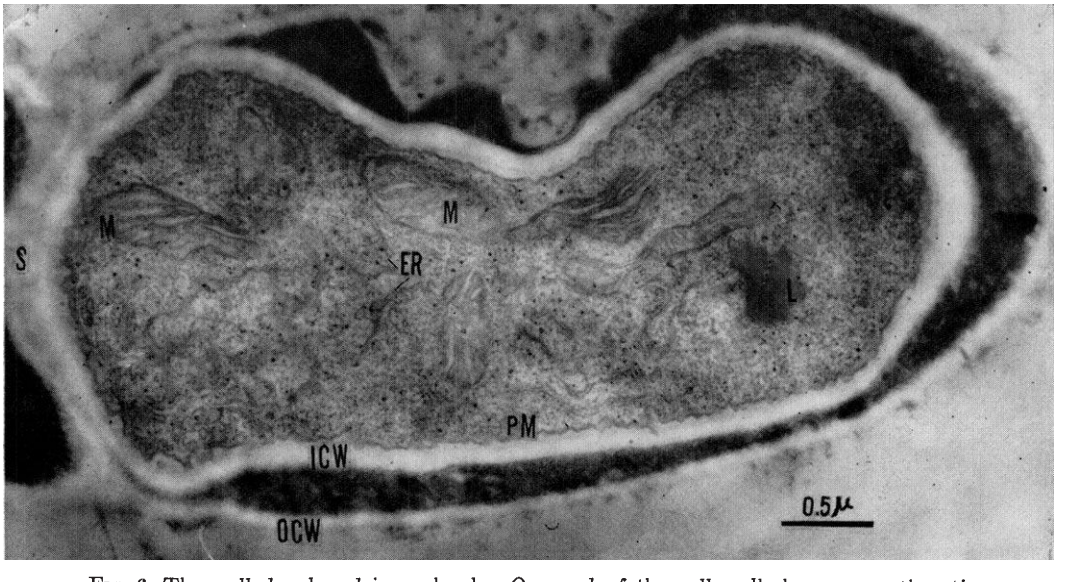

FIG. 6. The well developed inner hypha. One end of the cell wall shows a continuation with the septum (S) of the outer hypha, showing a process of coalescence.

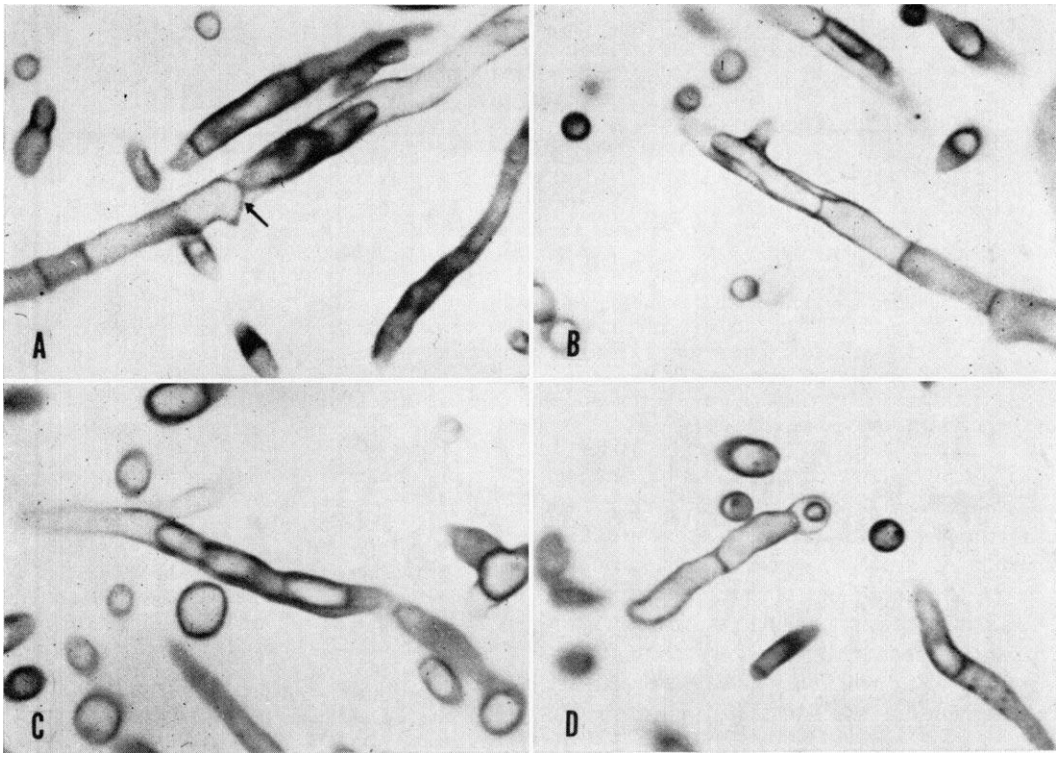

FIG. 7. Light microscopic findings of the double structure of hyphae. PAS stain, 1000×. In A, one end of the inner hypha is in contact with the septum of the outer hypha (indicated by an arrow).

Another mechanism of the development is shown in Figure 3. In some areas of the hypha, the cell loses its activity and cytoplasmic changes take place. Then the septum of the adjacent viable cell extends through the interior of the outer hypha, changing itself into the cell wall of the inner hypha. The cytoplasm of the outer hypha is concentrated and absorbed into the inner hypha and finally coalescence between the cell walls of the inner and the outer hypha results. In an electron microscopic study of *Keratinomyces ajelloi*, Meinhof (9) demonstrated that a septum was formed from the cell wall of a hypha. A reverse transformation may take place under certain culture conditions. According to Tsumagari (10) and Abe (11), the time at which we prepared specimens, i.e. 96 hours after culture, is the period when extension of the hypha and branch formation are very active.

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